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Anti-UBE3A Picoband™ Antibody (monoclonal, 8I3)

Catalog # ABO15002

Product Information

Application	WB, FC
Primary Accession	Q05086
Host	Mouse
Isotype	Mouse IgG2a
Reactivity	Human, Monkey
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-UBE3A Picoband™ Antibody (monoclonal, 8I3) . Tested in Flow Cytometry, WB applications. This antibody reacts with Human, Monkey.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	7337
Other Names	Ubiquitin-protein ligase E3A, 2.3.2.26, E6AP ubiquitin-protein ligase, HECT-type ubiquitin transferase E3A, Human papillomavirus E6-associated protein, Oncogenic protein-associated protein E6-AP, Renal carcinoma antigen NY-REN-54, UBE3A (HGNC:12496)
Calculated MW	100688
Application Details	Western blot, 0.25-0.5 □g/ml, Human, Monkey Flow Cytometry, 1-3 □g/1x10^6 cells, Human
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Clone Names	Clone: 8I3
Immunogen	E.coli-derived human UBE3A recombinant protein (Position: M1-E860).
Purification	Immunogen affinity purified.
Storage	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.

Protein Information

Name	UBE3A (HGNC:12496)

Function	E3 ubiquitin-protein ligase which accepts ubiquitin from an E2 ubiquitin-conjugating enzyme in the form of a thioester and transfers it to its substrates (PubMed:10373495, PubMed:16772533, PubMed:19204938, PubMed:19233847, PubMed:19325566, PubMed:19591933, PubMed:22645313, PubMed:24273172, PubMed:24728990, PubMed:30020076). Several substrates have been identified including the BMAL1, ARC, LAMTOR1, RAD23A and RAD23B, MCM7 (which is involved in DNA replication), annexin A1, the PML tumor suppressor, and the cell cycle regulator CDKN1B (PubMed:10373495, PubMed:19204938, PubMed:19325566, PubMed:19591933, PubMed:22645313, PubMed:24728990, PubMed:30020076). Additionally, may function as a cellular quality control ubiquitin ligase by helping the degradation of the cytoplasmic misfolded proteins (PubMed:19233847). Finally, UBE3A also promotes its own degradation in vivo. Plays an important role in the regulation of the circadian clock: involved in the ubiquitination of the core clock component BMAL1, leading to its proteasomal degradation (PubMed:24728990). Acts as transcriptional coactivator of progesterone receptor PGR upon progesterone hormone activation (PubMed:16772533). Acts as a regulator of synaptic development by mediating ubiquitination and degradation of ARC (By similarity). Required for synaptic remodeling in neurons by mediating ubiquitination and degradation of LAMTOR1, thereby limiting mTORC1 signaling and activity-dependent synaptic remodeling (By similarity). Synergizes with WBP2 in enhancing PGR activity (PubMed:16772533).
Cellular Location	Cytoplasm {ECO:0000250|UniProtKB:O08759}. Nucleus {ECO:0000250|UniProtKB:O08759}

Background

Ubiquitin-protein ligase E3A (UBE3A) also known as E6AP ubiquitin-protein ligase (E6AP) is an enzyme that in humans is encoded by the UBE3A gene. It is mapped to 15q11.2. This gene encodes an E3 ubiquitin-protein ligase, part of the ubiquitin protein degradation system. This imprinted gene is maternally expressed in brain and biallelically expressed in other tissues. Maternally inherited deletion of this gene causes Angelman Syndrome, characterized by severe motor and intellectual retardation, ataxia, hypotonia, epilepsy, absence of speech, and characteristic facies. The protein also interacts with the E6 protein of human papillomavirus types 16 and 18, resulting in ubiquitination and proteolysis of tumor protein p53. Alternative splicing of this gene results in three transcript variants encoding three isoforms with different N-termini. Additional transcript variants have been described, but their full length nature has not been determined.

Images

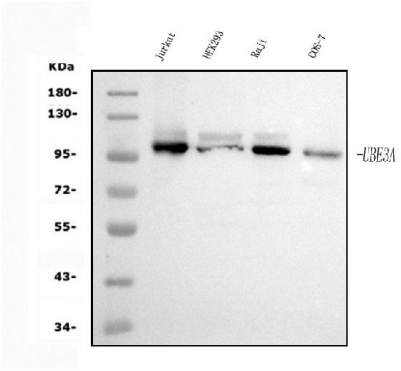


Figure 1. Western blot analysis of UBE3A using anti-UBE3A antibody (M00582-2).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours.
The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human Jurkat whole cell lysates,
Lane 2: human HEK293 whole cell lysates,
Lane 3: human Raji whole cell lysates,
Lane 4: monkey COS-7 whole cell lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes.
Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-UBE3A antigen affinity purified monoclonal antibody

(Catalog # M00582-2) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for UBE3A at approximately 100KD. The expected band size for UBE3A is at 100KD.

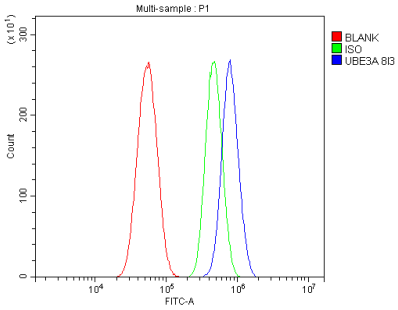


Figure 2. Flow Cytometry analysis of U87 cells using anti-UBE3A antibody (M00582-2).
Overlay histogram showing U87 cells stained with M00582-2 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-UBE3A Antibody (M00582-2, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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